PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	PREVALENCE AND INCIDENCE OF ANTIBODIES AGAINST SARS-COV-2 AMONG PRIMARY HEALTHCARE PROVIDERS IN BELGIUM DURING ONE YEAR OF THE COVID-19 EPIDEMIC: PROSPECTIVE COHORT STUDY PROTOCOL
AUTHORS	Adriaenssens, Niels; Scholtes, Beatrice; Bruyndonckx, Robin; Verbakel, Jan; De Sutter, An; Heytens, Stefan; Van Den Bruel, Ann; Desombere, Isabelle; Vandamme, Pierre; Goossens, Herman; Buret, Laetitia; Duysburgh, Els; Coenen, Samuel

VERSION 1 – REVIEW

REVIEWER	Westman, Gabriel Uppsala University, Department of Medical Sciences
REVIEW RETURNED	14-Sep-2021

GENERAL COMMENTS	Overall, this is a well-written study protocol but revisions are needed to enhance the utility and possibly also to modify the study design. 1) If the underlying objective is to analyse whether primary healthcare givers are at increased risk of SARS-CoV-2 infection, a control group matched for age and calender time seems highly relevant. Are there no data from healthy blood donors or similar to establish a basis for relative risk calculations? 2) Given the level of ambition for this study (N=5000), the statistical analysis plan should detail methods for primary and secondary hypotheres and how type 1-error will be managed. This could add substantial strength the the claims later made when results are available. 3) The authors should clarify what antibody assays that will be used, what antigens are targeted (nucleocapsid, spike or other) and antibody class (ie IgM och IgG). Based on this, clarification is needed on how humoral response from vaccination is diffrentiated from disease-related antibodies. 4) The authors should clarify why point-of-care (POC) antibody testing is of added clinical value. I understand the need for POC antigen or nuclein acid-based diagnostics showing ongoing infection where quick results could have implications in patient management, but do not understand what value is added by a quick (but possible less precise) result on antibody testing. The POC validation appears at least semi-promotional.

REVIEWER	Kristiansen, Marnar Fríðheim

	University of the Faroe Islands, Faculty of Health Sciences
REVIEW RETURNED	16-Sep-2021

GENERAL COMMENTS	This is a study protocol that sets out to prospectively investigate seroprevalence in a cohort of primary health care providers in Belgium. Further, they set out to assess the longevity of serological antibody response among seropositive individuals. They also aim to validate a point of care test for use in primary care settings. These are important research questions, and I look forward to reading their results when the studies are published. Below are my comments. On page 6, lines 15-16, the authors write: "seroprevalence studies in Iceland and Spain showed different levels of population antibody positivity, lasting up to 4 months in Iceland." This should rather be written as "at least 4 months" to avoid confusion. Regarding validation of the POCT. I worry that using the POCT that the authors plan to will not accurately depict waning immunity. It is not necessarily true that a negative POCT will accurately correspond with waning immunity. The authors plan to use a point of care test, with a reported sensitivity of 92.9% and specificity of 96.3%. Of these measures, the specificity is more concerning, as this specificity will yield multiple false-positive results. However, the sensitivity would feasibly also be lowest in participants with lower amounts of antibodies. The authors correctly will validate all positive by serum testing, which will provide valuable information for validating the POCT test and a better picture of the true seroprevalence in the cohort. However, this serological testing is only used once - at the beginning of the study. This means that results later in the study, where only POCT are used, will still be susceptible to these testing issues. This can, of course, be corrected in the final seroprevalence results by using these testing measures in the calculations. But this does not correct the results for waning immunity. Might I suggest that an additional serum sample is taken if serological status changes in a participant? If one participant initially is negative but later returns a positive POCT sample, they would be offered a serum sample. And if a participant initially is positive but later returns a negative POCT sample, this participant is also offered a serum sample. By analyzing serum samples for all participants with changed serological status, the issues with POCT sensitivity and specificity would be remedied. Alternatively, the authors could consider offering a serum sample at the final sample for all participants who had a change in serological status. It would be interesting to compare seroprevalence and severity amongst PHCP with the seroprevalence in the general population. It is not specifically mentioned in the protocol if they plan to do this, but as mentioned in the protocol, national seroprevalence studies have been performed on several occasions in Belgium, which would make these comparisons possible.

REVIEWER	Doganay, Levent Saglik Bilimleri Universitesi Umraniye Egitim ve Arastirma Hastanesi
REVIEW RETURNED	17-Sep-2021

GENERAL COMMENTS	This manuscript is a protocol of a study investigating seroprevalence of Covid-19 among primary health care providers (PHCP) in Belgium. Authors will use immunological serology-based point-of-care test (POCT) (from finger prick blood) to determine the antibody positivity. In a group of volunteers authors will collect blood samples to validate POCT results. Authors will follow up the cohort with questionnaires and follow up POCT for 12 months. At the end of study authors aim to find out prevalence of covid-19, seroprevalence of covid-19, duration of seropositivity, proportion of asymptomatic cases, risk factors for the disease among PHCPs. I recommend acceptance of this study protocol, I raise a minor issue that I am sure the authors have an explanation; At the moment vaccination rate (fully vaccinated) in Belgium is 72%. One can assume that this ratio is even higher among PHCPs. Covid-19 is also prevalent among PHCPs. So it is possible that authors will come across with volunteers who; 1- have already vaccinated 2- had infection and then vaccinated 3- vaccinated and then infected If the study has not been already conducted before mass vaccination in Belgium, authors need to have a plan to dissect the conditions above, they had better to discard volunteers who had infected and vaccinated before enrollment.

VERSION 1 – AUTHOR RESPONSE

Response to Reviewer comments:

Reviewer: 1
Dr. Gabriel Westman, Uppsala University
Comments to the Author:
Overall, this is a well-written study protocol but revisions are needed to enhance the utility and possibly also to modify the study design.
Response: Thank you for your kind feedback. Since the study has already started data collection – meanwhile we are preparing for the last originally planned testing time point – the possibilities to modify the study design are limited.

1) If the underlying objective is to analyse whether primary healthcare givers are at increased risk of SARS-CoV-2 infection, a control group matched for age and calender time seems highly relevant. Are there no data from healthy blood donors or similar to establish a basis for relative risk calculations?
Response: Thank you for this suggestion. As mentioned before, at this stage of the study it is hard to include a control group matched for age and calendar time. On the other hand, to establish a basis for relative risk calculation data from other relevant populations are available. As mentioned in the Introduction, for Belgium, Sciensano (the Belgian national scientific institute, www.sciensano.be) performs national seroprevalence studies of SARS-CoV-2 antibodies in several relevant populations besides primary health care providers, including schools, hospital personnel and nursing homes. The results of these seroprevalence studies are made publicly available at:

https://datastudio.google.com/embed/reporting/7e11980c-3350-4ee3-8291-3065cc4e90c2/page/ZwmOB. We have used a similar approach to compare risk of SARS-CoV-2 infection between primary health care providers and the general population in Flanders in "Mariën J, Ceulemans A, Bakokimi D, Lammens C, Ieven M, Heytens S, et al., Prospective SARS-CoV-2 cohort study among primary health care providers during the second COVID-19 wave in Flanders, Belgium, Family Practice, 2021;cmab094. DOI: 10.1093/fampra/cmab094". We have not included an additional objective regarding the comparison of the seroprevalence between primary health care providers and the general population in the revised version of this manuscript to adhere to the original protocol (registered at ClinicalTrials.gov). Instead we have added this extra statement to the 'Strengths and limitations of the study' statements:
• Regular online data collection provides timely data that can be compared to that of the general population and other population groups, e.g. health care workers in hospitals and nursing homes.

2) Given the level of ambition for this study (N=5000), the statistical analysis plan should detail methods for primary and secondary hypotheres and how type 1-error will be managed. This could add substantial strength the the claims later made when results are available.
Response: Thank you for referring to our ambition and additional suggestions. The sample size of 5000 primary health care providers (PHCPs) was based on consideration regarding the different objectives of the proposed study are explained in detail in the Methods section. Regarding the primary objectives, i.e. to assess 1. the prevalence and 2. the incidence of antibodies against SARS-CoV-2 among PHCPs in Belgium during a 12-month follow-up period, and most secondary objectives, we aimed for estimating proportions with a high precision rather than for hypothesis testing at a certain significance level. Regarding one of the secondary objectives, i.e. to assess the determinants (risk and predictive factors) of SARS-CoV-2 infection in PHCPs, a large sample size could indeed result in statistical significance of clinically insignificant (irrelevant) determinants. Hence, we will carefully consider the clinical relevance of any statistically significant determinants of SARS-CoV-2 infection in PHCPs.

3) The authors should clarify what antibody assays that will be used, what antigens are targeted (nucleocapsid, spike or other) and antibody class (ie IgM och IgG). Based on this, clarification is needed on how humoral response from vaccination is diffrentiated from disease-related antibodies.
Response: Thank you for this suggestion. Indeed, we should explicitly state in the manuscript text that the Orientgene® point-of-care test (POCT) targets IgM and IgG against the receptor binding domain (RBD) of SARS-CoV-2 and can therefore also provide valuable information in a vaccinated population. Although the POCT we use does not allow to differentiate between Ig response due to vaccination or to natural infection, which is not the objective of our study, information on the participants' vaccination status and the occurrence of COVID-19 infections is collected through the online follow-up questionnaire which is completed by the participants at each testing time point during follow-up.
We have added this information to the abstract and the Introduction of the revised version of the manuscript and added this extra statement to the 'Strengths and limitations of the study' statements:
• The POCT used targets IgM and IgG against the receptor binding domain of SARS-CoV-2 and can therefore also provide valuable information in a vaccinated population.

4) The authors should clarify why point-of-care (POC) antibody testing is of added clinical value. I understand the need for POC antigen or nuclein acid-based diagnostics showing ongoing infection where quick results could have implications in patient management, but do not understand what value is added by a quick (but possible less precise) result on antibody testing. The POC validation appears at least semi-promotional.
Response: Thank you for this comment. We consider the value of serological point-of-care tests (POCTs) in the context of surveillance rather than in the context of clinical practice in our study. In the context of seroprevalence studies, the seroprevalence in PHCPs and in nursing home residents and

staff is currently being assessed in collaboration with Sciensano with the same POCTs. Compared to our previous work mentioned before that used dried blood spots (DBS) to assess the prevalence and incidence of antibodies against SARS-CoV-2 among primary health care providers (PHCPs) in Flanders, the use of serological POCTs substantially improves the timeliness of the test results and allows the PHCPs to immediately check their results. Together with Sciensano, we believe it is important to assess the seroprevalence over time among PHCPs as they manage the vast majority of COVID-19 and other patients and therefore play an essential role in the efficient organisation of healthcare.
Regarding the validation of the POCT, we would like to emphasize that none of the authors have any conflict of interest as declared in the competing interests statement. The aim of the validation in this study is to validate the best of five POCTs independently validated by Sciensano in a laboratory setting, when this POCT is used by primary health care providers (PHCPs) in a primary care setting, i.e. to compare the sensitivity and specificity which are independently determined in laboratory conditions by Sciensano to the sensitivity and specificity of the POCT when used by PHCPs in real-life conditions. We purchased the POCTs used in our study and the company producing the test is not involved in the design, data collection, analysis, interpretation or publication of the results of our validation of their POCT.

Reviewer: 2
Dr. Marnar Fríðheim Kristiansen, University of the Faroe Islands, National Hospital of the Faroe Islands
Comments to the Author:
This is a study protocol that sets out to prospectively investigate seroprevalence in a cohort of primary health care providers in Belgium.
Further, they set out to assess the longevity of serological antibody response among seropositive individuals. They also aim to validate a point of care test for use in primary care settings.
These are important research questions, and I look forward to reading their results when the studies are published.
Response: Thank you for your kind feedback.

Below are my comments.
On page 6, lines 15-16, the authors write:
"seroprevalence studies in Iceland and Spain showed different levels of population antibody positivity, lasting up to 4 months in Iceland."
This should rather be written as "at least 4 months" to avoid confusion.
Response: Thank you for this suggestion. We have revised the manuscript accordingly.

Regarding validation of the POCT.
I worry that using the POCT that the authors plan to will not accurately depict waning immunity. It is not necessarily true that a negative POCT will accurately correspond with waning immunity.
The authors plan to use a point of care test, with a reported sensitivity of 92.9% and specificity of 96.3%. Of these measures, the specificity is more concerning, as this specificity will yield multiple false-positive results. However, the sensitivity would feasibly also be lowest in participants with lower amounts of antibodies. The authors correctly will validate all positive by serum testing, which will provide valuable information for validating the POCT test and a better picture of the true seroprevalence in the cohort.
However, this serological testing is only used once - at the beginning of the study. This means that results later in the study, where only POCT are used, will still be susceptible to these testing issues. This can, of course, be corrected in the final seroprevalence results by using these testing measures in the calculations. But this does not correct the results for waning immunity.

Might I suggest that an additional serum sample is taken if serological status changes in a participant? If one participant initially is negative but later returns a positive POCT sample, they would be offered a serum sample. And if a participant initially is positive but later returns a negative POCT sample, this participant is also offered a serum sample.
By analyzing serum samples for all participants with changed serological status, the issues with POCT sensitivity and specificity would be remedied.
Alternatively, the authors could consider offering a serum sample at the final sample for all participants who had a change in serological status.
Response: Thank you for these comments and valuable suggestions regarding the validation of the POCT. Since our study has already started data collection – meanwhile we are preparing for the last originally planned testing time point – the possibilities to modify the study design are limited.
We fully agree that the accuracy of the POCT is not perfect and that seroprevalence result can be corrected by the actual sensitivity and specificity of the POCT used. That is the reason our study aims to validate the best of five POCTs independently validated by Sciensano in a laboratory setting, when this POCT is used by primary health care providers (PHCPs) in a primary care setting, i.e. to assess the sensitivity and specificity of the POCT when used by PHCPs in real-life conditions (and compare it to the sensitivity and specificity independently determined in laboratory conditions by Sciensano).
Given the progress of the originally planned study and the start of the booster vaccination of PHCPs, it is no longer possible to provide all participants with study materials to collect a serum sample for a laboratory based serological testing if their serological status changes compared to an earlier testing time point. If this study is extended, we could consider implementing your suggestion. Meanwhile, we have added reduced POCT accuracy as limitation to the 'Strengths and limitations of the study' statements and replaced reference to OrientGene® by the information that the POCT used in this study targets IgM and IgG against the receptor binding domain of SARS-CoV-2 both in the Abstract and in the Introduction.
Meanwhile, it is important to stress that the objective of our study is to assess the prevalence and incidence of SARS-CoV-2 antibodies among a population group, i.e. PHCPs, which is different from assessing PHCPs' immunity status (waning immunity). To answer our study objective, we opted for a testing approach that is logistically easy to implement, has an acceptable cost, and has an, for this objective, acceptable sensitivity and specificity. Keeping in mind that no test has a 100% sensitivity and specificity, the estimated seroprevalence used for our sample size calculation and the reporting of the test sensitivity and specificity are important to put in context and be able to understand/interpret the seroprevalence results correctly.

It would be interesting to compare seroprevalence and severity amongst PHCP with the seroprevalence in the general population. It is not specifically mentioned in the protocol if they plan to do this, but as mentioned in the protocol, national seroprevalence studies have been performed on several occasions in Belgium, which would make these comparisons possible.
Response: Thank you for this suggestion. Data are indeed available to compare the seroprevalence amongst PHCP with the seroprevalence in the general population. As mentioned in the Introduction, for Belgium, Sciensano (the Belgian national scientific institute, www.sciensano.be) performs national seroprevalence studies of SARS-CoV-2 antibodies in several populations besides primary health care providers. The results of these seroprevalence studies are made publicly available at: https://datastudio.google.com/embed/reporting/7e11980c-3350-4ee3-8291-3065cc4e90c2/page/ZwmOB. We have used a similar approach to compare risk of SARS-CoV-2 infection between primary health care providers and the general population in Flanders in "Mariën J, Ceulemans A, Bakokimi D, Lammens C, Ieven M, Heytens S, et al., Prospective SARS-CoV-2 cohort study among primary health care providers during the second COVID-19 wave in Flanders, Belgium, Family Practice, 2021;cmab094. DOI: 10.1093/fampra/cmab094". We have not included an additional objective regarding the comparison of the seroprevalence between primary health care providers and the general population in the revised version of this manuscript to adhere to the original protocol

(registered at ClinicalTrials.gov). Instead we have added this extra statement to the 'Strengths and limitations of the study' statements:
• Regular online data collection provides timely data that can be compared to that in the general population and other population groups, e.g. health care workers in hospitals and nursing homes.

Reviewer: 3
Dr. Levent Doganay, Saglik Bilimleri Universitesi Umraniye Egitim ve Arastirma Hastanesi Comments to the Author:
Dear Editor,
This manuscript is a protocol of a study investigating seroprevalence of Covid-19 among primary health care providers (PHCP) in Belgium. Authors will use immunological serology-based point-of-care test (POCT) (from finger prick blood) to determine the antibody positivity. In a group of volunteers authors will collect blood samples to validate POCT results.
Authors will follow up the cohort with questionnaires and follow up POCT for 12 months. At the end of study authors aim to find out prevalence of covid-19, seroprevalence of covid-19, duration of seropositivity, proportion of asymptomatic cases, risk factors for the disease among PHCPs.
I recommend acceptance of this study protocol, I raise a minor issue that I am sure the authors have an explanation;

At the moment vaccination rate (fully vaccinated) in Belgium is 72%. One can assume that this ratio is even higher among PHCPs. Covid-19 is also prevalent among PHCPs. So it is possible that authors will come across with volunteers who;
1- have already vaccinated
2- had infection and then vaccinated
3- vaccinated and then infected
If the study has not been already conducted before mass vaccination in Belgium, authors need to have a plan to dissect the conditions above, they had better to discard volunteers who had infected and vaccinated before enrollment.

Response: Thank you for this comment and for your recommendation. Our study started data collection before mass vaccination in Belgium and is still ongoing. We are currently preparing for the last originally planned testing time point. Therefore, the possibilities to modify the study design are limited.
The point-of-care test (POCT) we use targets IgM and IgG against the receptor binding domain (RBD) of SARS-CoV-2 and can therefore also provide valuable information in a vaccinated population. Although this POCT does not allow to differentiate between Ig response due to vaccination or to natural infection, which is not the objective of our study, information on the participants' vaccination status and the occurrence of COVID-19 infections is collected through the online follow-up questionnaire which is completed by the participants each testing time point during follow-up.
Based on this information we will be able to address some, but not all, of the categories/conditions you mention. Because infections with SARS-CoV-2 often remain asymptomatic, infections will not always be identified by the information we collect through the questionnaires. Nevertheless, we want to stress again that this was not the objective of our study. However, based on POCT results and questionnaire information we can differentiate between the following: Before vaccination of the PHCPs all positive test could only be related to natural infection. After vaccination, positive tests could be related to both vaccination and to natural infection (both before and after vaccination).

VERSION 2 – REVIEW

REVIEWER	Kristiansen, Marnar Fríðheim University of the Faroe Islands, Faculty of Health Sciences
REVIEW RETURNED	21-Dec-2021

GENERAL COMMENTS	The authors have adequately addressed the comments and feedback and have stated the limitations needed for their methods. I have no further comments.